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HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

August 21, 2003

MEMORANDUM

SUBJECT: **2,4-DB [4-(2,4-Dichlorophenoxy)butyric acid]**. Chemical 030801. HED Review of Meat/Milk Analytical Method (860.1340) and Ruminant Feeding Study (860.1480).DP Barcode D240248 [MRIDs 44334701 through 44334705].

FROM: Danette Drew, Chemist
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THROUGH: Whang Phang, Branch Senior Scientist
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The 2,4-DB Task Force has submitted a ruminant feeding study (1997; MRID 44334705) and method validation data for an analytical method for determinating residues of 2,4-DB in beef tissues and milk (1997; MRID 44334704). These submissions are evaluated herein for their adequacy in fulfilling the residue chemistry data requirements for 2,4-DB reregistration.. The registrants also submitted three studies (1996, MRID 44334701; 1997, MRIDs 44334702 and 44334703) characterizing 2,4-DB, 2,4-DB glycine conjugate and other analytes of 2,4-DB for their use in GLP studies. These studies are briefly summarized here for informational purposes.

These data have been reviewed by Dynamac Corp. under contract to the Agency and have undergone secondary review in Reregistration Branch 3 of the Health Effects Division to reflect current policies.

RC
01/04

2,4-DB
PC Code 030801; Case 0196
(DP Barcode D240248)

Registrant's Response to Residue Chemistry Data Requirements

April 22, 1998

Contract No. 68-D4-0010

Submitted to:
U.S. Environmental Protection Agency
Arlington, VA

Submitted by:
Dynamac Corporation
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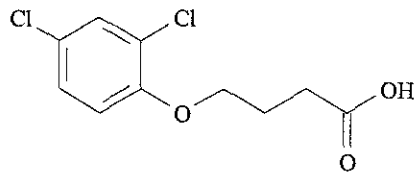
2,4-DB



PC Code 030801; Case 0196

DP Barcodes D240248

REGISTRANT'S RESPONSE TO RESIDUE CHEMISTRY DATA REQUIREMENTS

BACKGROUND

In response to the 2,4-DB Registration Standard, dated 2/1/88, the 2,4-DB Task Force (consisting of A.H. Marks and Co., Ltd., Aceto Agricultural Chemicals, Cedar Chemical Corporation, and Rhone-Poulenc Ag Company) submitted a ruminant feeding study (1997; MRID 44334705) and method validation data for an analytical method for determinating residues of 2,4-DB in beef tissues and milk (1997; MRID 44334704). These submissions are evaluated herein for their adequacy in fulfilling the residue chemistry data requirements for 2,4-DB reregistration. The Conclusions and Recommendations stated in this review pertain only to analytical methodology and the magnitude of the residue in livestock (ruminant) commodities.

The registrants also submitted three studies (1996, MRID 44334701; 1997, MRIDs 44334702 and 44334703) characterizing 2,4-DB, 2,4-DB glycine conjugate and other analytes of 2,4-DB for their use in GLP studies. These studies are briefly summarized in the analytical methods section of this report.

Tolerances for residues of 2,4-DB in/on plant commodities are currently expressed in terms of the combined residues of 2,4-DB [4-(2,4-dichlorophenoxy)butyric acid] and its metabolite 2,4-dichlorophenoxyacetic acid [40 CFR 180.331]. A tolerance of 0.2N ppm is currently established for alfalfa, clover, mint hay, peanuts, soybean seed and hay and birdsfoot trefoil. No tolerances have been established for 2,4-DB residues of concern in livestock commodities. The Pesticide Analytical Manual (PAM) Vol. II, lists Method I, a GC method with microcoulometric detection, for the enforcement of tolerances for 2,4-DB residues; this method is the PAM Vol. I method for chlorophenoxy acid residues in food.

The qualitative nature of the residue in plants is adequately understood based on metabolism studies with alfalfa, peanuts, and soybeans. The HED Metabolism Committee (D221954, D. Miller, 1/17/96) has determined that 2,4-DB *per se* is the only residue to be regulated in plants, provided that no additional uses on any human food items are sought. The qualitative nature of the residue in livestock is adequately understood based on metabolism studies with lactating goats and laying hens. The HED Metabolism Committee has determined that 2,4-DB *per se* is the only residue to be regulated in livestock commodities. However, in its review of the protocol for the ruminant feeding study, the Agency agreed that it would be appropriate "for the registrant to include a hydrolysis step in the method to hydrolyze the 2,4-DB glycine to parent 2,4-DB"(DP Barcode D224176, D. Miller, 5/7/96). In the ruminant metabolism study (DP barcodes D196291, D197243, and D197685, D. Miller, 1/26/95), 2,4-DB glycine accounted for 85%, 9.1% and 8.5% of the TRR (total radioactive residue) in goat milk, liver and kidney, respectively. Therefore, the registrants have included a hydrolysis step in the methods analyzing for 2,4-DB in liver, kidney and milk.

There are no established or proposed Codex MRLs for 2,4-DB residues. Therefore, there are no issues of compatibility with respect to U.S. tolerances and Codex MRLs.

CONCLUSIONS AND RECOMMENDATIONS

1a. The submitted GC/ECD method used from determining residues of 2,4-DB in ruminant tissues and milk are adequate. The validated limit of quantitation (LOQ) of 2,4-DB in tissues and milk is 0.05 ppm and 0.01 ppm, respectively. The limit of detection (LOD) is 0.03 ppm in tissue and 0.006 ppm in milk. Overall method recoveries from tissue fortified at 0.05-0.2 ppm and from milk fortified at 0.01-0.1 ppm were 63-119% for 2,4-DB and 70-114% for 2,4-DB glycine.

1b. The above GC/ECD method should be proposed as an enforcement method for determining 2,4-DB (free and conjugated) in livestock commodities. A method radiovalidation study has been submitted and is undergoing HED review (MRID 44997901).

2. The submitted storage stability data are adequate and indicate that 2,4-DB is stable in frozen liver (91 days), muscle (101 days), fat (105 days), kidney (119 days), and milk (116 days). These data support the maximum storage intervals from the ruminant feeding study of ~35 days observed for tissues and milk.

3a. Three groups of lactating dairy cows (3 cows/group) were dosed orally *via* capsules for 28 consecutive days with 2,4-DB at levels equivalent to 1.5, 4.5, and 15 ppm in the diet. These dose levels approximate 1x, 3x and 10x the theoretical dietary burden for beef (1.65 ppm) and dairy cattle (1.43 ppm).

3b. Residues of 2,4-DB were ≤0.01 ppm (LOQ) for all samples of milk at all dose levels. One sample each from the 1x (day 1) and 3x (day 21) dose levels bore residues at 0.01 ppm; residues of 2,4-DB in the remaining samples at these dose levels were <0.01 ppm. For the 10x dose group, residues of 2,4-DB in milk samples were <0.01 ppm with the exception of three samples. Two samples on day 3, and one sample on day 14 bore residues of 2,4-DB at 0.01 ppm.

3c. For all tissue matrices, residues of 2,4-DB were <0.05 ppm (LOQ) in samples from the 1x and 3x dose groups. One sample each of kidney and liver from the 10x dose group bore residues of 2,4-DB at 0.05 and 0.11 ppm, respectively. All other tissue samples from the 10x dose group were <0.05 ppm.

3d. The submitted ruminant feeding study is adequate, and indicates that residues of 2,4-DB may transfer to beef liver as a result of the current registered uses of 2,4-DB on livestock feedstuffs. The appropriate tolerance for 2,4-DB is 0.05 ppm (LOQ) in the liver of cattle, goats, horses, and sheep. There is no reasonable expectation of the transfer of residues of 2,4-DB from feed items to livestock meat, fat, kidney or milk; therefore, the current use of 2,4-DB with respect to these commodities should be classified as Category 3 under 40 CFR 180.6(a), and tolerances for residues of 2,4-DB in meat, fat, kidney and milk of cattle, goats, horses, and sheep is not required.

DETAILED CONSIDERATIONS

Residue Analytical Methods

The 2,4-DB task force submitted method validation data (1997, MRID 44334704) for a GC/ECD method for determining residues of 2,4-DB and its glycine conjugate in beef tissues and milk. The HED metabolism commitee (D221954, D. Miller, 1/17/96) has concluded that 2,4-DB *per se* is the residue of concern in livestock commodities. The registrant has also submitted data on the recovery of 2,4-DB glycine conjugate on account of its significant contribution to the TRR as determined in the ruminant metabolism study. In a metabolism study (DP barcodes D196291, D197243, and D197685, D. Miller, 1/26/95), 2,4-DB glycine accounted for 85%, 9.1% and 8.5% of the TRR in goat milk, liver and kidney, respectively. Therefore, a hydrolysis step was added to the methods analyzing for 2,4-DB in liver, kidney and milk to account for the conjugate. In conjunction with the ruminant feeding study discussed below (1997, MRID 44334705), the 2,4-DB Task Force submitted concurrent method recoveries for the same GC/ECD method described in the validation study. The method validation study and sample analyses for the current submission were performed by PTRL East, Inc., Richmond, KY. A brief description of each of the analytical methods submitted are provided in the following paragraphs.

Residues in muscle are extracted with acidified acetonitrile (ACN), centrifuged, filtered, and diluted with water. 2,4-DB Residues are sequentially partitioned into diethyl ether (Et_2O) then 0.1% NaOH. Residues are then concentrated, acidified to pH 2, and applied to C8 and C18 columns eluted with methyl (tert) butyl ether (MTBE) for cleanup. The residues in the eluate are concentrated, and methylated using boron trifluoride in methanol (BF_3/MeOH) prior to analysis by GC/ECD.

Residues in fat are extracted with hexane, partitioned into 0.1% NaOH, acidified (pH 2-3), and partitioned into Et_2O. Residues of 2,4-DB are then purified and determined using the same procedures described above for muscle.

Residues in kidney and liver are initially hydrolyzed in 4N HCl (reflux 2 hours). Hydrolyzed residues are then partitioned into ACN and cleaned up on a florisil column eluted with ACN. Residues in the eluate are partitioned into 1% NaOH, acidified (pH 2), concentrated, and partitioned into ethyl acetate:hexane (1:9,v/v). The organosoluble residues are then cleaned up on an alumina column eluted with MeOH:1% NaOH (1:9, v/v), acidified (pH 2), partitioned into MTBE, and concentrated. The 2,4-DB residues are then methylated with BF_3/MeOH prior to analysis by GC/ECD.

Samples of milk are hydrolyzed in 2N HCl (reflux 1 hour), and residues in the resulting hydrolysate are extracted with ACN. The ACN layer is removed and the purification and determination proceed as described above for kidney and liver.

The LOQ of 2,4-DB in beef tissues and milk is 0.05 ppm and 0.01 ppm, respectively. The LOD is 0.03 ppm in beef tissue and 0.006 ppm in milk.

For method validation and concurrent method recovery, control samples of each matrix were fortified with 2,4-DB at 0.05-0.20 ppm; liver, kidney and milk were also fortified with the 2,4-DB glycine conjugate at the same levels. Method recoveries are presented in Table 1. Overall method recoveries from the various matrices were 63-119% for 2,4-DB, and 70-114% for 2,4-DB glycine. Apparent residues of each analyte were below the LOQ for all control samples analyzed for each matrix. These data indicate that the GC/ECD methods described above are adequate for collecting data on residues of 2,4-DB and its glycine conjugate in meat and milk.

The above GC/ECD method should be proposed as an enforcement method for determining 2,4-DB in livestock commodities. A method radiovalidation study has been submitted and is undergoing HED review (MRID 44997901).

Table 1. Method validation and concurrent recoveries of 2,4-DB and 2,4-DB glycine from ruminant tissues and milk.

Matrix	Metabolite	Fortification levels (ppm)	Number of Samples	% Recovery
Method Validation (MRID 44334704)				
Fat	2,4-DB	0.05-0.20	19	68-117
Liver	2,4-DB	0.05-0.20	20	72-117
	2,4-DB glycine	0.05-0.20	4	70-91
Muscle	2,4-DB	0.05-0.20	18	70-108
Kidney	2,4-DB	0.05-0.20	19	63-109
	2,4-DB glycine	0.05-0.20	4	78-110
Milk	2,4-DB	0.01-0.10	11	71-108
	2,4-DB glycine	0.01-0.10	7	71-114
Concurrent Recoveries (MRID 44334705)				
Fat	2,4-DB	0.05-0.10	3	73-85
Liver	2,4-DB	0.05	2	77, 106
Muscle	2,4-DB	0.05-0.10	3	86-113
Kidney	2,4-DB	0.05-0.10	3	94-119
	2,4-DB glycine	0.10	1	95
Milk	2,4-DB	0.05-0.10	32	70-117
	2,4-DB glycine	0.05-0.10	4	82-108

In addition to the method validation and residue study, the registrants also submitted three studies (1996, MRID 44334701; 1997, MRIDs 44334702 and 44334703) characterizing 2,4-DB, 2,4-DB glycine conjugate and other analytes of 2,4-DB for their use in GLP studies. These studies are briefly summarized here for informational purposes. Using a variety of analytical techniques, 2,4-DB, 2,4-DB glycine, 2,4-D and 2,4-D phenol were characterized by physical appearance, melting point, elemental analysis, purity, and UV, MS and NMR spectrum. Chemical purity was determined by HPLC analysis. The purity of two 2,4-DB lot/batches analyzed (88/2016 and 01911LW) was 98.14% and 100%, respectively. Batch number 88/2016 (98.14% purity) was used in the ruminant feeding study for dosing and as an analytical reference standard; lot number 01911LW (purity 100%) was used strictly as a reference standard. Lot number P1018A-1 of 2,4-DB glycine, also used in the present feeding study as a reference standard, was analyzed and determined to be 99.81% pure. 2,4-D and 2,4-D phenol were characterized with a purity of 100% (lot numbers 12913TN and 09617KN), but were not used in the ruminant feeding study.

Storage Stability

In conjunction with the magnitude of the residue study (1997, MRID 44334705), the 2,4-DB Task Force submitted data on the frozen storage stability of 2,4-DB and 2,4-DB glycine residues in beef tissues and milk.

Milk samples were stored frozen for 1 - 36 days prior to extraction and analysis. Tissue samples were collected, separately bagged, placed in coolers on ice and transported to PTRL East, Inc., where the tissues were rinsed, chopped, and placed in frozen storage prior to processing. For processing in preparation for extraction, the frozen tissues were subsampled, homogenized with dry ice in a commercial food processor, and returned to frozen storage. Tissue samples were stored frozen for 12-34 days prior to extraction and analysis.

Control samples of tissue and milk were fortified with 0.5 ppm and 1.0 ppm of 2,4-DB, respectively, and the fortified samples were placed in frozen storage at an unspecified temperature. In addition, the stability of 2,4-DB glycine was tested using separate milk control samples fortified with 2,4-DB glycine at 1.0 ppm. Fortified samples of each matrix were analyzed on the day of fortification and again at ~1 month and at 3-4 months. For each matrix at each sampling interval, two freshly fortified controls were analyzed along with two stored fortified samples and a control sample using the adequate GC/ECD methods described above. Results of the analyses are presented in Table 2, and indicate that 2,4-DB is stable in frozen liver (91 days), muscle (101 days), fat (105 days), kidney (119 days), and milk (116 days and 139 days for 2,4-DB glycine).

The submitted storage stability data are adequate and support the frozen storage intervals (maximum of 36 days) depicted in the current feeding study.

Table 2. Storage stability of 2,4-DB and 2,4-DB glycine in milk and tissues fortified at 1.0 ppm and 0.5 ppm, respectively, and stored frozen at an unspecified temperature for up to 4 months.

Matrix	Storage Interval	Fortification Level	Percent Recovery [a]		
			Fresh Fortification	Stored Sample	Corrected Stored Sample [b]
2,4-DB					
Liver	0	0.5	81.6	84.3	103.3
	31	0.5	80.5	88.6	110.1
	91	0.5	73.2	83.0	113.4
Muscle	0	0.5	82.5	85.2	103.3
	35	0.5	81.8	92.5	113.1
	101	0.5	76.1	78.3	102.9
Fat	0	0.5	80.1	91.2	113.8
	47	0.5	85.6	62.8	73.4
	105	0.5	98.2	100.3	102.1
Kidney	0	0.5	79.1	81.5	103.0
	35	0.5	75.8	73.9	97.4
	119	0.5	87.4	101.6	116.2
Milk	0	1.0	75.1	74.7	99.4
	35	1.0	77.7	78.0	100.3
	116	1.0	94.6	81.3	86.0
2,4-DB glycine					
Milk	0	1.0	70.7	71.8	101.5
	39	1.0	41.7	51.3	123.2
	139 [c]	1.0	95.6	92.6	96.9

[a] Recovery values represent the mean of duplicate analyses.
[b] Represents the percent recovery of the stored sample corrected for the freshly fortified concurrent recovery.
[c] Recovery values are based on the one sample analysis.

Magnitude of the Residues in Meat and Milk

Ruminant Feeding Study.

Tolerances for residues of 2,4-DB in/on plant commodities are currently expressed in terms of the combined residues of 2,4-DB [4-(2,4-dichlorophenoxy)butyric acid] and its metabolite 2,4-dichlorophenoxyacetic acid [40 CFR 180.331]. The HED metabolism commitee (D. Miller, 1/17/96) has concluded that 2,4-DB *per se* is the residue of concern in livestock commodities. No tolerances have been established for 2,4-DB residues in livestock commodities.

In response to the Reregistration Standard, the 2,4-DB Task Force submitted a protocol (DP Barcode D224176, D. Miller, 5/7/96) for a ruminant feeding study, and the Agency calculated the maximum theoretical dietary burdens (MTDB) for beef and dairy cattle using the recently recommended tolerances for 2,4-DB on livestock feed items. The recommended tolerances were listed as follows: alfalfa forage (0.7 ppm), alfalfa hay (2 ppm), soybean seed (0.5 ppm), soybean forage (0.7 ppm), soybean hay (2 ppm), peanut hay (0.6 ppm), and peanut meal (0.05 ppm). In the protocol review, the calculated MTDB was 1.32 ppm for beef and dairy cattle. The calculated MTDB for beef and dairy cattle based on the recommended tolerances and the percent livestock diet described in the updated residue chemistry guidelines is 1.7 ppm and 1.4 ppm, respectively (Table 3).

Table 3. Calculation of maximum theoretical dietary burden for beef and dairy cattle.

Feedstuffs	% Dry Matter [a]	% Diet [a]	Tolerance (ppm) [b]	Dietary Contribution (ppm) [c]
Beef Cattle				
Alfalfa hay	89	70	2.0	1.57
Soybean seed	89	15	0.5	0.08
TOTAL BURDEN		85		1.65
Dairy Cattle				
Alfalfa hay	89	60	2.0	1.35
Soybean seed	89	15	0.5	0.08
TOTAL BURDEN		75		1.43

[a] Table 1, OPPTS GLN 860.1000.
[b] Recommended tolerances from ruminant study protocol review (DP Barcode D224176, D. Miller, 5/7/96).
[c] Contribution = [tolerance / %DM (if cattle)] X % diet).

The following is a review of the completed ruminant feeding study (1997; MRID 44232401) submitted by the registrants. The in-life phase of the study and sample analyses were conducted by PTRL East.

Three groups of lactating cows (3 cows/group) were dosed orally for 28 consecutive days with 2,4-DB at 29, 86, or 290 mg ai/cow/day, which is equivalent to 1.5, 4.5, and 15 ppm in the diet. These feeding levels approximate 1x, 3x and 10x the theoretical dietary burden for beef and dairy cattle (Table 3). One cow served as the control. Following a 1-week acclimation period, cows were dosed with capsules containing 2,4-DB using a balling gun once a day following the evening milking. Fresh batches of capsules for dosing were prepared each week. Capsules were not administered to the control animal.

Two extra capsules per dose group were prepared each week and analyzed by HPLC to verify the dosages. Analyses of these capsules indicated that the actual doses were ≥96% of the dose amounts predicted by gravimetric measurement.

Animals were observed throughout the study for mortality and moribundity. Feed consumption and milk production were recorded daily, and animals were weighed prior to dosing and at sacrifice. There was no apparent effect of 2,4-DB dosing on overall animal health, feed consumption, or milk production at any dose level.

Animals were milked twice a day, and milk samples were collected on Study Days 0 (pre-dosing), 1, 3, 7, 11, 14, 18, 21, 24, and 27. Milk samples for a given study day consisted of the morning (a.m.) sample and the evening (p.m.) sample. Subsamples of the a.m. and p.m. samples were composited based upon their proportion of the total daily production; milk samples were stored frozen for 1-36 days prior to extraction and analysis.

Control and treated cows were sacrificed within 20 hours of receiving the final dose, and carcasses were examined for gross pathology. No gross pathological findings were noted in any of the cows. Samples of kidney (both), liver, composite muscle (round and loin), and fat (perirenal and omental) were collected, bagged, placed in coolers on ice and transported to PTRL East, Inc., where the tissues were rinsed, chopped, and placed in frozen storage prior to processing. For processing prior to extraction, the frozen tissues were subsampled, homogenized with dry ice in a commercial food processor, and returned to frozen storage. The maximum frozen storage interval for tissue samples was 34 days (kidney).

For analysis of milk, a single sample from each cow was analyzed from study days 0 (pre-dose), 1, 3, 7, 11, 14, 18, 21, and 27 for each dose group. For analysis of residues in tissues, a single sample of each matrix was analyzed for each cow in each dose group. Results from the analyses of milk and tissues are presented in Tables 4 and 5, respectively. Residue values are reported in 2,4-DB equivalents.

Residues of 2,4-DB and 2,4-DB glycine were determined using the GC/ECD method described above. The validated limit of quantitation (LOQ) of 2,4-DB in tissues and milk is 0.05 ppm and 0.01 ppm, respectively. The limit of detection (LOD) is 0.03 ppm in tissue and 0.006 ppm in milk. Concurrent method recoveries from control samples of each matrix fortified with 2,4-DB and kidney and milk samples fortified with 2,4-DB glycine conjugate at 0.05-0.10 ppm were 70-119%. Apparent residues of each analyte were below the LOQ for all control samples analyzed for each matrix.

Residues of 2,4-DB were at or below the LOQ for all samples of milk at all dose levels. One sample each from the 1x (day 1) and 3x (day 21) dose levels bore residues at the LOQ (0.01 ppm); residues of 2,4-DB in the remaining samples at these dose levels were <LOQ. For the 10x dose group, residues of 2,4-DB in milk samples were <LOQ with the exception of three samples. Two samples on day 3, and one sample on day 14 bore residues of 2,4-DB at 0.01 ppm.

For all tissue matrices, residues of 2,4-DB were <LOQ (0.05 ppm) in samples from the 1x and 3x dose groups. One sample each of kidney and liver from the 10x dose group bore residues of 2,4-DB at 0.05 and 0.11 ppm, respectively. All other tissue samples from the 10x dose group were <LOQ.

The submitted ruminant feeding study is adequate. The data indicate that residues of 2,4-DB may transfer to beef liver as a result of the current registered uses of 2,4-DB on livestock feedstuffs. The appropriate tolerance for 2,4-DB (free and conjugated) is 0.05 ppm in the liver of cattle, goats, horses, and sheep. There is no reasonable expectation of the transfer of residues of 2,4-DB from feed items to livestock meat, fat, kidney or milk; therefore, the current use of 2,4-DB with respect to these commodities should be classified as Category 3 under 40 CFR 180.6(a), and tolerances for residues of 2,4-DB in meat, fat, kidney and milk of cattle, goats, horses, and sheep is not required.

Table 4. Residues 2,4-DB in the milk dairy cattle dosed at 1x, 3x, and 15x the maximum theoretical dietary burden for 28 days.

Study Day	2,4-DB Residues (ppm)[a] in milk		
	Dose Level		
	1.5 ppm (1x)	4.5 ppm (3x)	15 ppm (10x)
0	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01
1	<0.01, **0.01**, <0.01	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01
3	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01	**0.01, 0.01**, <0.01
7	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01
11	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01
14	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01	**0.01**, <0.01, <0.01
18	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01
21	<0.01, <0.01, <0.01	<0.01, <0.01, **0.01**	<0.01, <0.01, <0.01
24	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01
27	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01

[a] Data represent the analysis of a single sample from each cow and are expressed in 2,4-DB equivalents.
[b] Value in parenthesis is the dose expressed on the reviewer's calculated maximum theoretical dietary burden.

Table 5. Residues of 2,4-DB in tissues of dairy cattle dosed at 1x, 3x, and 10x the maximum theoretical dietary burden for 28 days.

Matrix	Residues (ppm) [a]		
	Dose Level		
	1.5 ppm (1x) [b]	4.5 ppm (3x) [b]	15 ppm (10x) [b]
Muscle	<0.05, <0.05, <0.05	<0.05, <0.05, <0.05	<0.05, <0.05, <0.05
Fat	<0.05, <0.05, <0.05	<0.05, <0.05, <0.05	<0.05, <0.05, <0.05
Liver	<0.05, <0.05, <0.05	<0.05, <0.05, <0.05	<0.05, <0.05, 0.11
Kidney	<0.05, <0.05, <0.05	<0.05, <0.05, <0.05	<0.05, <0.05, 0.05

[a] Data represent the analysis of a single sample from each cow and are expressed in 2,4-DB equivalents.

[b] Value in parenthesis is the dose expressed on the reviewer's calculated maximum theoretical dietary burden.

AGENCY MEMORANDA CITED

DP Barcode: D196291, D197243, and D197685
Subject: 2,4-DB. (030801) Nature of the Residue in Alfalfa, Peanuts, Soybeans, Ruminants, and Poultry and Field Trial Analytical Method in Soybeans. Proposed Analytical Enforcement Method for Plant Commodities.
From: D. Miller
To: J. Coombs
Dated: 1/26/95
MRIDs: 42965901, 43009801, 43033801-3, and 430339-01

DP Barcode: D224176
Subject: 2,4-DB (030019) Review of Registrant's Proposed Protocol for Ruminant Feeding Study.
From: D. Miller
To: P. Deschamp
Dated: 5/7/96
MRIDs: None

DP Barcode: D221954
Subject: 2,4-DB. Results of the 1/16/96 Meeting of the HED Metabolism Committee. 2,4-DB
From: D. Miller
To: HED Metabolism Committee
Dated: 1/17/95
MRIDs: None

MASTER RECORD IDENTIFICATION NUMBERS

44334701 King, D. (1996) Characterization of 2,4-Dichlorophenoxybutyric Acid (2,4-DB), 2,4-Dichlorophenoxyacetic Acid (2,4-D) and 2,4-Dichlorophenol (2,4-D Phenol): Lab Project Number: 1013: 1885. Unpublished study prepared by PTRL East, Inc. 28 p.

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cc: D. Drew, RF
RDI: J.Morales (8/5/03); W. Phang (8/21/03)

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